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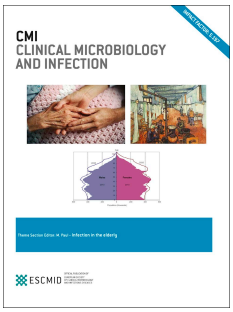

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Hepatitis C virus: life cycle in cells, infection and host response, and analysis of molecular markers influencing the outcome of infection and response to therapy

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Abstract

Hepatitis C virus (HCV) is a major global health burden accounting for around 170 million chronic infections worldwide. Since its discovery, which dates back to about 30 years ago, many details of the viral genome organization and the astonishing genetic diversity have been unveiled but, owing to the difficulty of culturing HCV *in vitro* and obtaining fully susceptible yet immunocompetent *in vivo* models, we are still a long way from the full comprehension of viral life cycle, host cell pathways facilitating or counteracting infection, pathogenetic mechanisms *in vivo*, and host defenses. Here, we illustrate the viral life cycle into cells, describe the interplay between immune and genetic host factors shaping the course of infection, and provide details of the molecular approaches currently used to genotype, monitor replication *in vivo*, and studying the emergence of drug-resistant viral variants.

(137 words)

Keywords: Hepatitis C virus; Cell culture-derived HCV; Pathogenesis; Host response; Broadly-reactive neutralizing antibodies; Genotype; Quantitative molecular assays; Drug resistance; Monitoring drug resistance; Direct antiviral agents.

HCV: A momentous virus for Virology

The Hepatitis C virus (HCV) was identified in 1989. This year marks a departure from traditional virology, based on isolation, cultivation, and biochemical studies, to modern virology that uses molecular biology and biotechnology to discover, characterize, and monitor viruses. HCV is indeed the first infectious agent discovered thanks to molecular biology techniques that, owing to the difficulty to replicate the virus *in vitro*, have been extensively used to define the molecular aspects of HCV biology.

Today HCV is making history again. Recently developed direct-acting antivirals (DAAs) eliminate infection in over 90% treated individuals and are changing the idea that antivirals, in general, can at most block viral replication and slow disease progression. Poorly tolerated interferon (IFN)-based therapeutic regimens are being rapidly replaced with IFN-free DAA regimens, and tissue damage in patients with advanced stages of disease is stabilized and possibly reversed [1]. Further, detailed study of HCV's dependence on host factors has permitted development of host-directed antiviral therapies. This review provides an overview of some key aspects of viral interaction at cellular and host levels and illustrates current methods to monitor viral replication and genotyping with particular emphasis on fundaments and recent clinical findings important to determine susceptibility or resistance to DAAs.

HCV life cycle and host-cell interactions *in vitro*

HCV belongs the Flaviviridae, a large family of enveloped, single-stranded RNA viruses that is organized into the genera Hepacivirus, Flavivirus, Pestivirus, and Pegivirus [2], and that includes many viruses transmitted by arthropods and growing matter of health concern [3]. The HCV life cycle is only partly understood; difficulties in establishing an *in vitro* model of replication and the complex network of cell surface molecules used to mediate viral entry

have delayed comprehension of various molecular mechanisms [4, 5]. Briefly, as shown in Figure 1, the HCV virion circulates in the bloodstream either as free-particle or surrounded by host low-density lipoproteins [6], attaches onto the target cell membrane by sequential binding of various receptor molecules, and enters into the cell by a clathrin-mediated endocytosis process. Disruption of the viral capsid in the endocytic compartment releases the 9.6 kb single-stranded RNA genome of positive polarity into the cytoplasm. The RNA genome is then directly translated at the rough endoplasmic reticulum (ER) in a single polyprotein precursor of about 3,000 amino acid residues that is eventually cleaved by cellular and viral proteases into ten mature products [7, 8]. These proteins, enlisted in the order they are encoded, include the structural core and envelope glycoproteins E1 and E2, and the following nonstructural proteins: p7 viroporin and nonstructural protein 2 (NS2) that participate in virus assembly and release; NS3 and NS4A, the protease complex that, as described in Chapters 3 and 4 of this Theme Section, is actively targeted by the protease inhibitor class of DAAs; NS4B, a membrane-associated protein that mediates virus–host interactions; NS5A, a zinc-binding and proline-rich hydrophilic phosphoprotein involved in HCV RNA replication and targeted by NS5A inhibitor DAAs; and NS5B, the RNA-dependent RNA polymerase targeted by nucleoside and non-nucleoside polymerase inhibitor DAAs. New virions are assembled in an ER-derived compartment and released by exocytosis following a Golgi-dependent secretory pathway. Along this process the virus undergoes maturation and becomes surrounded by endogenous lipoproteins that, as described below, are believed to help immune escape [4, 5]. Binding to host lipoproteins and envelope without clearly discernable surface features confer to HCV virions low buoyant density and broad size range (40–80 nm diameter) [5].

Lack of a reliable *in vitro* method to study HCV replication was due to scarce adaptability of primary hepatic cells to *in vitro* propagation, no availability of viral isolates

adapted to *in vitro* culture, and large use of cell-to-cell transmission to disseminate infection to neighbor cells. This mechanism has complicated identification of cellular receptors necessary for viral entry and is believed to facilitate immunological escape, virus persistence, and resistance to DAAs [9-12]. Also, receptor usage appears to depend upon cell type and infection via free-particle or cell-to-cell transmission [4, 13].

The cell culture derived HCV (HCVcc) and the HCV trans-complemented particles (HCV_{TCP}) are among the most used methods to study replication of HCV replication *in vitro* [4]. HCVcc uses JFH1, a HCV genotype 2a strain isolated from a Japanese patient with fulminant hepatitis and replicates in Huh-7, a human cell line from hepatocellular carcinoma [14, 15]. HCVcc generates infectious virus and, using either native or inter-genotype recombinant JFH1 variants, has allowed identifying some HCV entry factors, defining virion structure and biochemical properties, and testing DAA potency. HCV_{TCP}, described in detail elsewhere [16, 17], employs pseudotyped HCV virions generated in packaging cells transfected with viral proteins provided by different constructs. HCV_{TCP} can be theoretically obtained from any isolate but support only single-round infection and are unable to spread.

HCVcc, HCV_{TCP}, and basically all *in vitro* methods use Huh-7 cells that, although permissive to HCV replication, differ from primary hepatocytes for different restriction mechanisms, diverse localization of HCV receptors, and absence of the cell polarity observed in hepatic tissue. As a result, viral entry, assembly, release, and cell-to-cell spread observed *in vivo* is not completely reproduced *in vitro* [5]. HepG2 cell clones and hepatoma cells derived from primary hepatocytes are permissive to HCV replication *in vitro* and should allow better understanding of virus-host cell interplay. On this regard, a growing body of evidence shows that host genetics impact disease progression, immune response, and antiviral therapy [18, 19]. Nucleotide polymorphism of IFN and IFN-stimulated genes, for instance, strongly correlates with disease and therapy outcome to the point that host-targeting antivirals have

become an attractive field of drug research [20].

The natural history of infection

An estimated 130-200 million people worldwide are persistently infected with HCV, up to 4 million new infections occur annually, and the majority of infected persons are unaware of their infection status [21-23]. HCV spreads primarily through percutaneous contact with infected blood. Before identification, many people became HCV-infected as a result of unsafe injection practices, blood or blood products. Blood testing greatly reduced the risk of iatrogenic exposure in developed countries; however, unsafe medical procedures remain an important source of new infections particularly in resource-poor settings. In many developed countries, most infections now occur amongst people who use injected or intranasal drugs. Specific populations, notably human immunodeficiency virus (HIV)-positive men who have sex with men, are at increased risk of sexual transmission. Vertical transmission and transmission through piercing or tattooing are also possible [23, 24].

HCV causes hepatic inflammation and fibrosis that may progress sub-clinically over decades. Long-term sequelae include cirrhosis, end-stage liver disease, and hepatocellular carcinoma (HCC). In the Western world, chronic HCV infection is the leading indication for liver transplant and the leading cause of end-stage liver disease, HCC, and liver-related death [23, 25, 26]. Individual outcomes are highly variable, with many patients experiencing minimal changes while others progress rapidly [23]. Fibrosis progression is uneven and may accelerate with longer duration of infection; comorbid conditions such as HIV infection accelerate disease progression [22]. A large meta-analysis reported the risk of cirrhosis at 7-18% after 20 years and 41% after 30 years of infection [27]. Cirrhotic patients are at high risk for hepatic decompensation (27.7-39.5% risk over five years) and HCC (2.8-7.4% in the first year, and 8-16.1% over five years) [28]. Factors that increase the risks of fibrosis, cirrhosis,

and HCC include male sex, increasing age, alcohol use, and HIV co-infection.

Most infected individuals have no symptoms during the acute infection, although 15-30% may experience non-specific symptoms [22]. Because most acute HCV infections are sub-clinical, it is difficult to calculate how many patients clear HCV without treatment [22, 23]. This typically occurs in the first six months after exposure and is estimated at between <20% and about 50% in different populations [23]; spontaneous clearance is affected by host genetics, race, age, sex, and comorbidities such as HIV [29-32].

The host response and the outcome of HCV infection

Innate immunity

Soon after establishing infection in hepatic foci, HCV undergoes an exponential "ramp-up" phase of replication [33]; the rate of increase decreases abruptly when cells in the liver express a host of IFN-stimulated genes (ISGs) that limit HCV replication and spread [34]. Innate immunity is a first line of defense against HCV infection [35] and stimulates adaptive immunity. HCV RNA binds to retinoic acid-inducible gene I, activating mitochondrial antiviral signaling (MAVS) proteins; double-stranded RNA bound to Toll-like receptor-3 induces signaling via TIR domain-containing adaptor inducing IFN-β (TRIF). Both pathways activate NFκB and IRF3 translocation to the nucleus. Here, they promote expression of IFNs and ISGs to inhibit viral replication, plus proinflammatory cytokines and chemokines to recruit and activate immune cells. HCV's NS3-4A protease specifically cleaves MAVS and TRIF to dampen IFN induction [36, 37]. HCV may use several additional strategies to reduce innate antiviral responses (reviewed in [36, 37]). ISG expression in HCV-infected hepatocytes, even those with evidence of MAVS cleavage, demonstrates that these mechanisms do not completely abolish innate immunity [38, 39]. Hepatocytes preferentially express IFN-λ following HCV infection [40]. Dendritic cells, Kupffer cells, and other

nonparenchymal cells also recognize viral molecular patterns, contributing to IFN and cytokine production and response without themselves harboring replicating HCV [34, 36].

Role of adaptive immunity in the outcome of infection

Innate antiviral responses can limit HCV replication and spread, but rarely mediate elimination of infection without action by the adaptive immune response. Indeed, a hallmark of HCV control is the arrival in the liver of T-lymphocytes that produce IFN-γ. Elevated blood levels of transaminases, indicating hepatocyte cell death, are seen as viral loads decline. Chronic infection is defined as infection lasting more than 6 months; spontaneous clearance is rare but not unheard of after this point. Some key immunologic features of spontaneous clearance and chronic infection are summarized in Table 1.

In resolving infections, adaptive immunity rapidly mounts a cell-mediated response targeting multiple HCV epitopes and high-titer, broadly-reactive neutralizing antibodies (bNAbs) [41, 42]. By targeting multiple epitopes, T-lymphocytes reduce viral opportunities for immune escape. HCV's error-prone replication strategy permits rapid evolution, and immune responses select for variants that escape recognition. Some immune escape mutations are not tolerated because they impair viral fitness [43]. A second characteristic of effective anti-HCV immunity is the preservation of polyfunctional T-lymphocyte activity. $CD8^+$ T-lymphocytes (and other immune cells) are dependent on help from HCV-specific $CD4^+$ T-lymphocytes; these are readily detected early after HCV infection regardless of its outcome. In resolving infection, HCV-specific $CD4^+$ T-lymphocytes support $CD8^+$ T-lymphocyte survival, proliferation, and antiviral activity. Effector T-lymphocytes accumulate in the liver and slow HCV replication through cytokine release (IFN-γ, TNF-α) and killing of infected cells. T-lymphocyte responses persist long after the virus has been cleared, and mediate protection, albeit imperfect, against chronic infection in subsequent exposures. As

regards humoral immunity, bNAbs binding viral E2 and, to a lesser extent, E1 glycoproteins are produced at high titer and within the first few months of infection. This results in blocking HCV infection of its target cells. Key viral neutralization targets – i.e. conserved domains required for hepatocyte infection - are hidden behind glycans, lipoproteins, and hypervariable decoy domains. bNAbs bind the essential domains rather than the decoys [9, 44]. bNAbs may contribute to clearance because HCV must continuously infect new target cells to maintain even an established infection [45].

In contrast to the broad and sustained T-lymphocyte responses in resolving HCV infection, T-lymphocytes in persisting HCV infection may target a more limited set of epitopes; often, an initially broad response narrows. Thus, fewer viral sequence changes are required for immune escape. HCV that persists in chronic infection often shows evidence of immune-mediated selection for variants that avoid recognition by $CD8^+$ T-lymphocytes [34]. Where viral epitope sequences remain unchanged, HCV-specific T-lymphocyte responses are characterized by progressive loss of function. $CD4^+$ T-lymphocyte responses fail through a mechanism independent of epitope escape [34, 42]. $CD8^+$ T-lymphocytes, lacking $CD4^+$ T-lymphocyte help, lose effector functions, express markers associated with exhaustion, and stop proliferating. Inflammatory T-lymphocytes, frequently not HCV-specific, infiltrate the liver and may mediate tissue damage [46]. Finally, patients with chronic infection also express neutralizing antibodies, but these may arise later and may be isolate-specific, often targeting hypervariable epitopes with high potential for immune escape [9, 34].

Role of host genetics in the outcome of infection

HCV infection is more likely to persist in people bearing a set of unfavorable polymorphisms in the IFN-λ locus; the same alleles are associated with failure of IFN-α-based antiviral therapy [40]. High ISG expression in the infected liver is also a poor

prognostic indicator for IFN-α-based HCV treatment [47]. The cluster of IFN-λ locus polymorphisms includes a frame-shift in the IFN-λ4 gene; the favorable allele abolishes IFN-λ4 protein expression [40]. It has been proposed that IFN-λ4 might regulate hepatocyte IFN responsiveness, perhaps through negative feedback mechanisms. Alternatively, IFN-λ4 (or a linked polymorphism) may support prolonged innate immune activation and thereby interfere with maturation of an adaptive immune response [48]. Whether IFN-λ acts strictly via innate immunity, or influences adaptive immunity, is uncertain. Additional important polymorphisms are in the HLA locus. These may influence the outcome of infection through selection of immunodominant epitopes [43], cross-reactive responses [49], and interactions with natural killer cells [50].

Importance of viral load and genotyping in the outcome of infection and therapy

HCV viral load

Monitoring of the course of infection and therapeutic response is based on HCV RNA measurement in plasma or serum of patients and, under anti-HCV treatment, is aimed at optimizing therapy duration, and prompting early discontinuation to prevent potential side effects and reduce unnecessary costs. Baseline viral load, extent and sharpness of viremia decay in the early phases of treatment (4 and 12 weeks), and undetectable HCV RNA at the end of treatment represent key parameters guiding IFN-treatment.

With DAAs and according to current guidelines, HCV viral load monitoring during therapy remains crucial for patient management with regard to futility rules and assessment of therapy efficacy [51-53]. In addition, due to the rapid decay expected in almost all patients treated with DAAs, HCV RNA monitoring is also used to assess patient adherence.

The methods to measure HCV viral load have greatly evolved since their initial

establishment. Today automated real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and transcription-mediated amplification (TMA) platforms from different vendors are widely used throughout western countries (Table 2) [54,56]. These systems accurately quantify HCV RNA within a broad linear range, with a lower limit of detection (LLOD) sometimes even below the lower limit of quantification (LLOQ).

Despite good inter-assay agreement of linear range, there are important differences concerning LLOD, LLOQ and low HCV RNA concentration, which are crucial for clinical evaluation. Since decision to prolong or stop DDA therapy is often taken with a single measurement, accurate quantification is important. To reduce uncertainty, several strategies have been devised. For instance, with the Roche High Pure System/COBAS TaqMan assay virological response has been set at 25 IU/ml, the LLOQ of this assay. Depending on their LLOQ, other assays use lower levels [52-55]. Thus, the threshold and the number of patients achieving virological response may differ depending on the assay and influence clinical management [56-62]. For instance, patients receiving first generation DAA (Telaprevir) combined with PegIFN and RBV and with HCV RNA undetectable at week 4 were used to identify patients eligible for shortened treatment (24 vs 48 weeks) [63]. Based on this criterion, in the OPTIMIZE study, for instance, 34% or 72% of patients would have been eligible for shorter treatment if HCV RNA was measured with Abbott RealTime or Roche High Pure System/COBAS TaqMan [59]. Thus, current guidelines recommend considering assay performances and performing virological monitoring in the same laboratory for patient management [51, 61].

As mentioned, HCV must continuously infect new target cells to maintain even an established infection [45]. It is assumed therefore that clearance from each infected cell occurs when viral replication has been halted for a sufficient time. However, even when HCV RNA is not detected with the most sensitive assay, it is possible that minute, undetected

amounts of virus are still present. This “residual viremia”, firstly demonstrated for HIV with highly sensitive tests developed *ad hoc*, with enhanced sensitivity, may indicate suboptimal treatment and enhanced risk of virological failure [64]. To prevent this occurrence in HCV, ultrasensitive versions using increased input sample volume and modified calibration curve have been developed from commercial assays [65]. Preliminary data on patients receiving first generation DAA-based therapy suggest that early achievement of HCV RNA values undetectable with such ultrasensitive tests is predictive of sustained virological response [66]. Further studies are warranted to determine whether assays with enhanced sensitivity are necessary for clinical management of patients treated with new DAAs.

HCV core antigen (HCV Ag) is also gaining importance for effective monitoring of new generation DAAs. Architect HCV Core Antigen Test, one of most used systems, quantitates down to 0.06 pg/mL HCV Ag, roughly corresponding to 700 IU/mL HCV RNA. This marker proved useful in IFN-based therapy [67, 68] and good predictor of sustained virological response in first generation DAA-based therapy [69, 70]. This analysis is less expensive and time consuming compared to molecular assays, and should be considered whenever high analytical sensitivity is not a must [68, 69].

HCV genotyping

HCV shows enormous genomic sequence variability. Currently there are seven confirmed genotypes that are organized in 67 confirmed, 20 provisionally assigned, and 21 unassigned subtypes; median variability among genotypes and subtypes is approximately 33%, and 10%, respectively [71]. Because type and duration of treatment also depends on the genotype, HCV genotyping is mandatory in patients eligible for antiviral therapy [72]. Further, owing to important barriers to resistance, genotyping is also important for DAA-based therapies. As long as highly and broadly effective all oral combination therapies will be

available, it is conceivable the importance of HCV genotyping will decline in the future [72, 73].

Sequencing of conserved genomic regions is the gold standard for HCV genotyping, but this method is limited to high-level laboratories; reverse hybridization or genotype-specific real-time RT-PCR are easier-to-perform and routinely used but, particularly first generation assays that targeted the 5' untranslated region (5'UTR), can misclassify isolates especially at subtype level. A consistent improvement has been achieved by including coding regions in other targets, e.g. NS5B and core protein, possessing non-overlapping sequences amongst genotypes and subtypes [73].

Analysis of HCV resistance

Because of high replication rate and no proofreading activity of the viral polymerase, HCV is highly variable and each possible single mutation and combination of mutations may arise every day in a given infected individual. Genome plasticity and drug-driven selection create the conditions for the emergence of resistant variants [33] and, as a consequence, most mutations associated with resistance are located within the drug target regions (Figure 2) [74-76]. Despite the reduced fitness, such variants rapidly overgrow wild-type viruses and during this process they may accumulate additional, fitness-restoring, mutations [76]. Single drug, drug family and genotype/subtype often influence the emerging mutations. In turn, each DAA class displays different genetic barrier to resistance and cross-resistance between drugs inhibiting NS3 protease and NS5A is frequently observed.

Direct sequencing of PCR products has been the method of choice for investigating the presence of mutations conferring antiviral resistance. Unfortunately, this approach analyzes the predominant species and, as observed with HIV-1, variants below 20% may escape detection despite being crucial during the initial phases of resistance development [78]. Next

generation sequencing (NGS) detects and quantitates variants present at frequencies as low as 0.5% [79] and, therefore, permits early detection of resistance mutations, definition of their kinetics, and progressive disappearance after treatment suspension [80-83]. Although there are no commercial kits, many laboratories analyze NS3-4A, NS5A, and NS5B regions with NGS but its use in clinical practice is still limited and requires expert guidance for interpretation.

Natural variants carrying resistance-associated mutations have been repeatedly found by conventional and NGS analyses but, with the exception of searching for Q80K mutation in patients infected with genotype 1a and to be enrolled for treatment with Simeprevir, there is no agreement on the usefulness of performing resistance testing before DAA-based therapy [84-86]. Even the use of resistance testing in failing cases is questioned since: *i.* The emergence of resistant variants does not account for all virological failures; *ii.* The resistant variants rapidly decline (but do not always disappear) after stopping treatment [86, 87]; *iii.* The different classes and combination of drugs at disposal enormously increase the genetic barrier to resistance.

According to most recent guidelines and since NS5A resistance mutations can persist for over two years post-treatment [87], resistance testing is recommended for patients who need urgent treatment and have failed previous treatment with NS5A inhibitors. In contrast, the utility of routine testing for NS5B mutations is questionable. Resistance to nucleotide analogues targeting NS5B (i.e. Sofosbuvir) is rare and S282T, the only mutation (so far) associated with Sofosbuvir resistance, confers fitness disadvantage. As mentioned, however, unfit variants continue to evolve during treatment and select for compensatory mutations that counterbalance fitness loss.

Conclusions

Understanding the HCV life cycle and host factors that hinder or allow persistence of infection is crucial to develop post-exposure and prophylactic measures for the global control of HCV. Only a fraction of the global patient population has been diagnosed and, in spite of aforementioned remarkable advances in antiviral therapy, less than 1% has been treated [88-90] and those who have been treated remain vulnerable to re-infection [91]. Therapy alone is therefore not sufficient to eliminate the global burden of HCV infection and chronic liver disease in the near future. In contrast, individuals who have mounted a successful immune response against HCV have a substantial degree of protection upon subsequent exposures. This suggests that effective immunological strategies to protect against persistent infection are feasible [42, 89] and likely capable to face HCV's tremendous genetic diversity [71].

Transparency declaration

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Legend to figures

Figure 1. The HCV replication cycle. The seven steps of the viral life cycle, indicated in the white boxes, are the following: Attachment, the viral particle, surrounded with lipoproteins, binds the target cells by interacting with several receptors some, most of which shown in figure, considered essential other accessory; Entry: following attachment, the virus enters through clathrin-mediated endocytosis; Uncoating: the cellular and viral membranes fuse and the capsid is disorganized with a process triggered by the low pH of the endosome. After uncoating the positive-strand RNA genome is released into the cytoplasm; Translation: the genomic RNA is directly translated in a polyprotein precursor that is then cleaved into single proteins by both host and viral proteases; Replication: the non-structural proteins and some host factors form a replication complex that synthesized multiple copies of the HCV RNA genome via a minus-strand replicative intermediate; Assembly and maturation: packaging of viral progeny takes place in the endoplasmic reticulum from which the virion acquires the envelope with E1 and E2 glycoproteins. Maturation and association with endogenous lipoproteins to form lipoviral particles immediately follow; Release: virions are released from the cells most likely by exocytosis or transmitted to other cells via a cell-free mechanism.

Figure 2. A schematic of the mutations conferring resistance to NS3, NS5A and NS5B inhibitors. Numbers refer to the amino acid positions correlated to resistance in various HCV genotypes.

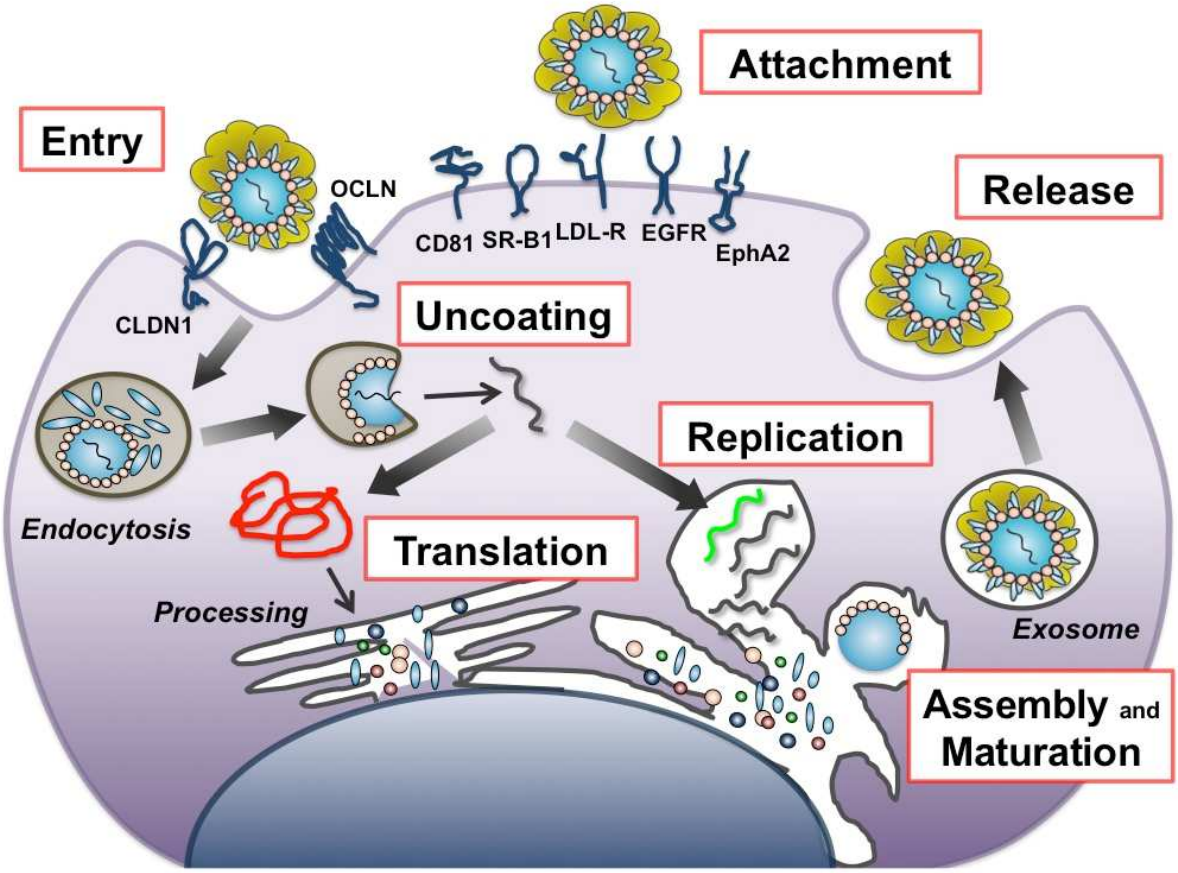
Table 1. Immunologic characteristics of resolving and persisting HCV infections

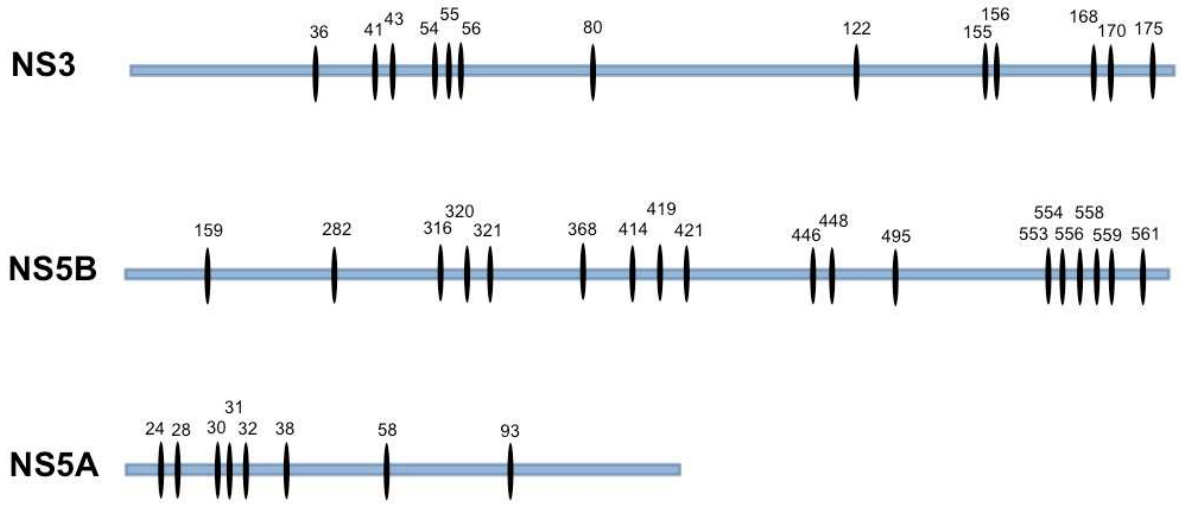
	Resolving infection	*Persisting infection*
T-cell responses	Broadly focused: limits viral escape options	Restricted number of epitopes facilitates viral escape
Duration of T-cell response	Rapid, sustained even after clearance	Wanes over time
HCV specific CD4+ T-cells	Persist	Disappear
HCV-specific CD8+ T-cells	• Proliferate and expand • Polyfunctional: produce IFNγ, TNFα • Express perforin	• Lose proliferative capacity • Fewer polyfunctional cells • Express markers of exhaustion
Neutralizing antibody	• Early • High titer • Broadly-reactive	• Isolate-specific • Viral sequence evolution outpaces antibody reactivity
IFNλ SNPs		
rs12979860	C	T
rs368234815	TT	ΔG
rs8099917	T	G

Table 2. Overview and features of automated assays marketed and licensed to quantitate HCV RNA in plasma or serum samples

Test name	*Manufacturer*	*Technology used*	*Level of automation*	*LLOD* [a] *(IU/ml)*	*LLOQ* [b] *(IU/ml)*	*Linear range (IU/ml)*
Artus Hepatitis C QS-RGQ	Qiagen	Real time RT-PCR	Moderate; extraction and amplification /detection in separate instruments; a few manual steps	15	20	up to $1.77x10^7$
CobasAmpliprep/ CobasTaqMan v2.0	Roche	Real time RT-PCR	High; extraction and amplification /detection in separate instruments; sample loading can be fully automated	15	15	up to 1.7×10^8
Abbott Real*Time* HCV	Abbott	Real time RT-PCR	High; extraction from primary tube; amplification/detection in a separate instrument; a few manual steps	12	12	up to $1.0x10^8$
Versant HCV RNA 2.0	Siemens	Real time RT-PCR	High; extraction from primary tube; amplification/detection in a separate instrument	15	15	up to 1.0×10^8
Aptima HCV Quant Dx Assay	Hologic	TMA [c]	Fully automated; extraction/amplification /detection all in one instrument, starting from primary tube)	4	12	up to $1.0x10^8$

[a] Lower limit of detection;
[b] Lower limit of quantification;
[c] Transcription-mediated amplification.


Attachment
Entry
OCLN
CD81
SR-B1
LDL-R
EGFR
EphA2
Release
CLDN1
Uncoating
Replication
Endocytosis
Translation
Processing
Exosome
Assembly and Maturation


NS3
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